

Dkt. 53801/JPW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Nika Adham, et al.
Serial No.: 09/116,676 Examiner: G. Draper
Filed: July 16, 1998 Group Art Unit: 1647
For: DNA ENCODING A HUMAN OB RECEPTOR (OB-RE) AND
USES THEREOF

1185 Avenue of the Americas
New York, New York 10036

#12
BQS
7/6/9

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

**DECLARATION OF NIKA ADHAM, BETH BOROWSKY,
NIGEL LEVENS, AND RADEK C. SKODA
UNDER 37 C.F.R. §1.131**

We, Nika Adham, Beth Borowsky, Nigel Levens, and Radek C. Skoda, hereby declare as follows:

1. We conceived of the invention claimed in the above-identified patent application, i.e., a process for determining whether a chemical compound specifically binds to a soluble polypeptide with specific properties as recited in claim 224 as amended in the Amendment filed concurrently with the filing of the Declaration (the "Binding Assay").

2. Prior to December 31, 1996, a Binding Assay was performed by Noel Boyle under the direction and supervision of coinventor Nika Adham in the United States at the laboratories of Synaptic Pharmaceutical Corporation, an assignee of record of the subject application. Copies of pages 189 and 190 of Noel Boyle's notebook number 11914 and pages 2-7 of Noel Boyle's notebook number 11915 detailing the performance of such a Binding Assay for determining whether leptin specifically binds to human Ob-Re, a soluble polypeptide as recited in claim 224 are attached hereto as **Exhibit A**. Although the dates have been redacted from these

EXHIBIT 2

Nika Adham, et al.
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Page 2

notebook pages, all dates are prior to December 31, 1996. Thus, at least one embodiment of the invention claimed was reduced to practice in the United States prior to December 31, 1996.

3. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1/29/01
Date

Nika Adham
Nika Adham

1/30/01
Date

Beth Borowsky
Beth Borowsky

5/18/01
Date

N. Levens
Nigel Levens

4/9/01
Date

R. Skoda
Radek C. Skoda

Project N

Book No.

TITLE

Leptin Soluble membranes

2

D: 125-I

Fr m Page USER: 3

COMMENT:

PRESET TIME : 1.00
DATA CALD : CPM H# : NO SAMPLE REPEATS: 1 PRINTER
COUNT BLANK : NO IC# : YES REPLICATES : 1 RS232
TWO PHASE : NO AGC : NO CYCLE REPEATS : 1
SCINTILLATOR: LIQUID LUMEX: NO LOW SAMPLE REJ: 0
LOW LEVEL : NO HALF LIFE CORRECTION DATE: none

ISOTOPE 1: -125I- XERROR: 2.00 FACTOR: 1.000000 BKG. SUB:

SAM NO	POS	TIME MIN	IC#	125I CPM	XERROR	LUMEX %	ELAPSED TIME
1	**1	1.00	4.785	2278.01	4.19	0.01	1.29
2	**2	1.00	5.337	762.01	7.25	0.02	2.62
3	**3	1.00	4.913	2074.06	4.39	0.01	3.97
4	**4	1.00	4.881	1816.07	4.69	0.01	5.34
5	**5	1.00	4.830	1701.08	4.85	0.01	6.67
6	**6	1.00	4.737	12115.13	4.35	0.01	8.02
7	**7	1.00	4.802	1761.13	4.77	0.01	9.39
8	**8	1.00	5.166	1416.12	5.31	0.01	10.74
9	**9	1.00	5.067	11375.13	5.39	0.01	12.09
10	**10	1.00	5.319	823.09	6.97	0.02	13.45
11	**11	1.00	5.427	749.09	7.31	0.03	14.81
12	**12	1.00	5.166	661.08	7.78	0.03	16.15
13	**13	1.00	4.810	2520.34	3.98	0.00	17.52
14	**14	1.00	5.642	688.10	7.62	0.02	18.87
15	**15	1.00	4.991	1902.30	4.59	0.01	20.22
16	**16	1.00	4.869	1859.31	4.64	0.01	21.60
17	**17	1.00	5.028	1882.34	4.61	0.01	22.96
18	**18	1.00	4.901	1900.36	4.59	0.01	24.30
19	**1	1.00	4.927	2042.41	4.43	0.01	25.77
20	**2	1.00	5.037	1492.32	5.18	0.01	27.12
21	**3	1.00	5.180	1179.26	5.82	0.01	28.47
22	**4	1.00	5.407	930.22	6.56	0.02	29.85
23	**5	1.00	5.645	777.19	7.17	0.03	31.20
24	**6	1.00	5.477	833.21	6.93	0.01	32.55
25	**7	1.00	4.896	2788.74	3.79	0.01	33.92
26	**8	1.00	5.175	2653.74	3.88	0.01	35.27
27	**9	1.00	4.842	2310.67	4.16	0.01	36.62
28	**10	1.00	4.901	2386.72	4.09	0.00	38.01
29	**11	1.00	4.972	2022.63	4.45	0.01	39.35
30	**12	1.00	4.852	1964.63	4.51	0.01	40.71
31	**13	1.00	5.096	2824.94	3.76	0.01	42.07
32	**14	1.00	5.028	2105.72	4.36	0.01	43.42
33	**15	1.00	5.034	1995.71	4.48	0.01	44.77
34	**16	1.00	5.152	1807.66	4.70	0.01	46.16
35	**17	1.00	5.238	1874.70	4.62	0.01	47.51
36	**18	1.00	4.993	2026.78	4.44	0.01	48.85
37	**1	1.00	5.635	787.31	7.13	0.02	50.32
38	**2	1.00	5.818	789.32	7.12	0.01	51.67
39	**3	1.00	5.614	682.29	7.66	0.02	53.02
40	**4	1.00	5.521	806.35	7.04	0.02	54.40
41	**5	1.00	5.520	660.29	7.78	0.02	55.75
42	**6	1.00	5.739	718.32	7.46	0.02	57.10
43	**7	1.00	5.784	724.34	7.43	0.01	58.47
44	**8	1.00	5.552	725.34	7.43	0.02	59.82
45	**9	1.00	5.613	725.35	7.43	0.02	61.17
46	**10	1.00	5.828	587.29	8.25	0.02	62.55

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EXHIBIT A

Nika Adham, et al.

Serial No.: 09/116,676

Filed: July 16, 1998

Leplin soluble membranes

TITLE	SAM	POS	TIME	IC#	125I	LUMEX	ECLIPSED
Fr m P	NO		MIN		CPM	%	TIME
	47	**11	1.00	5.951	790.40	7.12	63.90
	48	**12	1.00	5.913	696.36	7.58	65.25
	49	**13	1.00	5.789	456.24	9.37	66.64
	50	**14	1.00	5.281	848.46	6.87	67.97
	51	**15	1.00	5.938	523.29	8.75	69.34
	52	**16	1.00	5.143	551.31	8.52	70.70
	53	**17	1.00	5.821	517.30	8.80	72.05
	54	**18	1.00	5.782	542.32	8.59	73.40
	55	**1	1.00	6.896	577.34	8.33	74.89
	56	**2	1.00	6.307	428.26	9.67	76.24
	57	**3	1.00	5.909	533.33	8.66	77.59
	58	**4	1.00	5.996	493.31	9.01	78.95
	59	**5	1.00	6.075	521.33	8.76	80.30
	60	**6	1.00	5.425	345.22	10.77	81.65
	61	**7	1.00	5.346	684.45	7.65	83.04
	62	**8	1.00	6.001	757.51	7.27	84.39
	63	**9	1.00	5.880	755.51	7.28	85.72
	64	**10	1.00	5.927	788.55	7.12	87.10
	65	**11	1.00	5.825	901.63	6.66	88.45
	66	**12	1.00	5.973	797.57	7.08	89.81
	67	**13	1.00	6.264	807.58	7.04	91.19
	68	**14	1.00	6.368	765.56	7.23	92.54
	69	**15	1.00	6.138	801.60	7.07	93.89
	70	**16	1.00	6.385	766.58	7.23	95.25
	71	**17	1.00	6.255	787.60	7.13	96.60
	72	**18	1.00	5.925	830.65	6.94	97.95
	73	**1	1.00	6.061	732.58	7.39	99.42
	74	**2	1.00	5.934	738.59	7.36	100.77
	75	**3	1.00	5.994	703.57	7.54	102.12
	76	**4	1.00	5.710	657.54	7.80	103.50
	77	**5	1.00	6.291	503.42	8.92	104.85
	78	**6	1.00	6.667	456.39	9.37	106.20
	79	**7	1.00	6.552	466.40	9.26	107.57
	80	**8	1.00	6.639	494.43	9.00	108.92
	81	**9	1.00	6.056	860.75	6.82	110.27
	82	**10	1.00	5.902	726.65	7.42	111.65
	83	**11	1.00	6.462	808.73	7.04	113.00
	84	**12	1.00	6.495	644.59	7.88	114.35
	85	**13	1.00	6.222	771.71	7.20	115.72
	86	**14	1.00	6.174	739.69	7.36	117.07
	87	**15	1.00	6.219	644.61	7.88	118.42
	88	**16	1.00	6.132	540.52	8.61	119.80
	89	**17	1.00	6.709	394.38	10.08	121.15
	90	**18	1.00	6.737	545.53	8.57	122.50
	91	**1	1.00	6.458	624.62	8.01	123.97
	92	**2	1.00	6.216	568.57	8.39	125.32
	93	**3	1.00	6.198	728.73	7.41	126.67
	94	**4	1.00	6.151	763.78	7.24	128.05
	95	**5	1.00	6.245	883.91	6.73	129.40
	96	**6	1.00	6.383	787.82	7.13	130.75
	97	**7	1.00	6.123	5482.76	2.70	132.12
	98	**8	1.00	6.846	916.97	6.61	133.47
	99	**9	1.00	6.432	5008.37	2.83	134.82
	100	**10	1.00	6.311	4978.39	2.84	136.21
	101	**11	1.00	6.532	4710.15	2.92	137.56
	102	**12	1.00	6.668	4465.94	2.99	138.91
	103	**13	1.00	6.867	3962.42	3.18	140.27
	104	**14	1.00	6.903	2948.32	3.69	141.62

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Project N

Loglin Soluble membranes

PAGE: 1

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From P	SAM NO	POS	TIME MIN	IC#	125I		LUMEX %	ELAPSED TIME	TOT SAM NO
					CPM	%ERROR			
	105	**15	1.00	6.749	1720.96	4.82	0.01	142.99	163
	106	**16	1.00	6.994	1274.46	5.61	0.02	144.36	164
	107	**17	1.00	7.078	1013.17	6.27	0.01	145.70	165
	108	**18	1.00	6.841	989.16	6.36	0.01	147.05	166
	109	**1	1.00	6.536	3233.82	3.52	0.00	148.54	167
	110	**2	1.00	6.808	862.03	6.82	0.02	149.89	168
	111	**3	1.00	6.791	4667.62	2.93	0.00	151.24	169
	112	**4	1.00	6.839	3698.49	3.29	0.00	152.61	170
	113	**5	1.00	6.664	4233.19	3.08	0.00	153.96	171
	114	**6	1.00	6.871	3384.18	3.44	0.00	155.31	172
	115	**7	1.00	6.886	3795.73	3.25	0.00	156.69	173
	116	**8	1.00	7.171	2439.07	4.05	0.00	158.04	174
	117	**9	1.00	7.104	1767.24	4.76	0.01	159.39	175
	118	**10	1.00	6.970	1076.38	6.10	0.01	160.76	176
	119	**11	1.00	7.187	864.11	6.81	0.01	162.10	177
	120	**12	1.00	6.686	984.28	6.38	0.02	163.46	178
	121	**13	1.00	6.759	5354.02	2.74	0.00	164.84	179
	122	**14	1.00	6.855	4320.72	3.04	0.00	166.19	180
	123	**15	1.00	6.895	3913.22	3.20	0.00	167.54	181
	124	**16	1.00	6.852	4462.00	3.00	0.00	168.91	182
	125	**17	1.00	6.848	4448.03	3.00	0.00	170.26	183
	126	**18	1.00	6.895	4898.69	2.86	0.00	171.61	184
	127	**1	1.00	6.836	5364.39	2.73	0.00	173.09	185
	128	**2	1.00	7.120	5314.38	2.75	0.00	174.44	186
	129	**3	1.00	6.866	4718.60	2.91	0.00	175.79	187
	130	**4	1.00	6.750	4763.72	2.90	0.00	177.16	188
	131	**5	1.00	6.787	4473.36	2.99	0.00	178.51	189
	132	**6	1.00	6.598	5404.74	2.72	0.00	179.86	190
	133	**7	1.00	7.420	963.39	6.45	0.01	181.24	191
	134	**8	1.00	7.475	847.23	6.88	0.02	182.59	192
	135	**9	1.00	7.183	865.27	6.80	0.02	183.94	193
	136	**10	1.00	7.585	809.19	7.04	0.02	185.30	194
	137	**11	1.00	7.372	835.24	6.93	0.02	186.65	195
	138	**12	1.00	7.245	905.36	6.65	0.02	188.01	196
	139	**13	1.00	7.717	829.25	6.95	0.01	189.39	197
	140	**14	1.00	7.334	823.25	6.98	0.01	190.74	198
	141	**15	1.00	7.186	878.34	6.75	0.01	192.09	199
	142	**16	1.00	7.266	807.24	7.04	0.01	193.45	200
	143	**17	1.00	7.436	993.54	6.35	0.01	194.80	201
	144	**18	1.00	6.908	1018.59	6.27	0.01	196.15	202
	145	**1	1.00	6.312	2363.72	4.12	0.00	197.64	203
	146	**2	1.00	6.683	803.27	7.06	0.01	198.99	204
	147	**3	1.00	6.538	2331.72	4.15	0.01	200.34	205
	148	**4	1.00	6.341	2644.25	3.89	0.00	201.70	206
	149	**5	1.00	6.488	2583.18	3.94	0.00	203.06	207
	150	**6	1.00	6.486	2725.44	3.83	0.01	204.41	208
	151	**7	1.00	6.632	2159.54	4.31	0.01	205.79	209
	152	**8	1.00	6.725	1890.12	4.60	0.01	207.14	210
	153	**9	1.00	6.534	1441.39	5.27	0.01	208.49	211
	154	**10	1.00	6.915	949.59	6.50	0.01	209.85	212
	155	**11	1.00	6.459	804.35	7.06	0.02	211.20	213
	156	**12	1.00	6.557	774.31	7.16	0.01	212.55	214
	157	**13	1.00	6.619	2376.05	4.11	0.01	213.94	215
	158	**14	1.00	6.603	796.37	7.09	0.01	215.29	216
	159	**15	1.00	6.418	2617.52	3.91	0.00	216.64	217
	160	**16	1.00	6.540	2429.22	4.06	0.00	218.00	218
	161	**17	1.00	6.346	2521.41	3.99	0.00	219.36	219
	162	**18	1.00	6.273	2504.40	4.00	0.01	220.71	220

Witness:

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Optic obse

OB-Re
optical
medium

T=4780

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Regule med
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Regule med
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TITLE

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Project No.

Rank No.

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SAM NO	POS	TIME MIN	IC#	125I		LUMEX %	ELAPSED TIME																
				CPM	%ERROR																		
163	**1	1.00	6.481	2297.06	4.18	0.00	222.19																
164	**2	1.00	6.173	1735.09	4.81	0.01	223.54																
165	**3	1.00	6.487	1296.32	5.56	0.01	224.89																
166	**4	1.00	6.372	990.79	6.36	0.01	226.27																
167	**5	1.00	6.611	859.56	6.83	0.01	227.62																
168	**6	1.00	6.341	825.51	6.97	0.01	228.97																
169	**7	1.00	6.310	2527.64	3.98	0.01	230.34																
170	**8	1.00	6.347	2490.60	4.01	0.00	231.69																
171	**9	1.00	6.477	2621.87	3.91	0.00	233.04																
172	**10	1.00	6.502	2564.79	3.95	0.01	234.42																
173	**11	1.00	6.368	2810.28	3.78	0.00	235.77																
174	**12	1.00	6.265	2798.28	3.78	0.01	237.12																
175	**13	1.00	6.484	2657.05	3.88	0.00	238.49																
176	**14	1.00	6.390	2885.51	3.73	0.00	239.84																
177	**15	1.00	6.518	2926.62	3.70	0.01	241.19																
178	**16	1.00	6.521	2830.47	3.76	0.01	242.57																
179	**17	1.00	6.437	2753.35	3.82	0.01	243.92																
180	**18	1.00	6.506	2573.03	3.95	0.01	245.27																
181	**1	1.00	6.389	728.43	7.42	0.01	246.74																
182	**2	1.00	7.048	714.41	7.49	0.02	248.09																
183	**3	1.00	6.528	740.47	7.36	0.02	249.44																
184	**4	1.00	6.786	789.58	7.12	0.01	250.82																
185	**5	1.00	7.000	765.54	7.24	0.02	252.17																
186	**6	1.00	6.569	774.56	7.19	0.01	253.52																
187	**7	1.00	6.837	705.43	7.54	0.02	254.89																
188	**8	1.00	6.982	780.59	7.17	0.02	256.24																
189	**9	1.00	6.584	705.45	7.54	0.02	257.59																
190	**10	1.00	6.770	756.56	7.28	0.01	258.95																
191	**11	1.00	6.566	777.61	7.18	0.03	260.32																
192	**12	1.00	6.929	759.58	7.26	0.02	261.65																
193	**13	1.00	7.145	10135.24	1.99	0.00	263.04																
194	**14	1.00	7.162	2546.36	3.97	0.01	264.39																
195	**15	1.00	7.004	9852.86	2.02	0.00	265.74																
196	**16	1.00	6.960	9620.47	2.04	0.00	267.11																
197	**17	1.00	7.184	9693.73	2.03	0.00	268.46																
198	**18	1.00	7.138	9917.31	2.01	0.00	269.81																
199	**1	1.00	7.065	9112.69	2.10	0.00	271.29																
200	**2	1.00	7.036	6454.02	2.49	0.00	272.64																
201	**3	1.00	7.113	4094.94	3.13	0.01	273.99																
202	**4	1.00	6.945	3018.62	3.64	0.00	275.36																
203	**5	1.00	7.512	2743.05	3.82	0.00	276.71																
204	**6	1.00	6.900	2571.70	3.95	0.00	278.05																
205	**7	1.00	6.937	9689.57	2.03	0.00	279.44																
206	**8	1.00	7.075	2111.72	4.36	0.01	280.79																
207	**9	1.00	7.205	9980.43	2.00	0.00	282.14																
208	**10	1.00	7.198	10414.52	1.96	0.00	283.51																
209	**11	1.00	6.945	9862.38	2.02	0.00	284.86																
210	**12	1.00	6.996	9835.42	2.02	0.00	286.21																
211	**13	1.00	7.020	9442.63	2.06	0.00	287.59																
212	**14	1.00	7.087	6677.37	2.45	0.00	288.94																
213	**15	1.00	7.195	3916.06	3.20	0.00	290.29																
214	**16	1.00	7.005	2781.46	3.80	0.00	291.65																
215	**17	1.00	7.170	2736.39	3.83	0.00	293.01																
216	**18	1.00	7.380	2782.52	3.80	0.00	294.36																
217	**1	1.00	7.183	10229.11	1.98	0.00	295.84																
218	**2	1.00	7.209	9885.40	2.01	0.00	297.19																
219	**3	1.00	7.344	10359.64	1.97	0.00	298.54																
220	**4	1.00	7.347	10335.69	1.97	0.00	299.91																

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Project N _____
B k N _____

TITLE SPA LGPT in Soluble Membranes.

From Page N _____

SPA -

Auerbach kit
proximity assay

Plate 1 - hOB-Re ++

Plate 2 - hOB-Re ++ mock

Plate 3 - [Cos 7] hOB-Re, # B025 P91 (optimum medium) 1:2

Plate 4 - [Cos 7] hOB-Re, # B025 P91 (normal medium) 1:2

Plate 5 - [Cos 7] hOB-RB, # B07 P55

1ml = 2.4 (1:3)

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TITLE SPALEPTIN SOLUBLE MEMBRANES

Project No. _____

B k N. _____

7

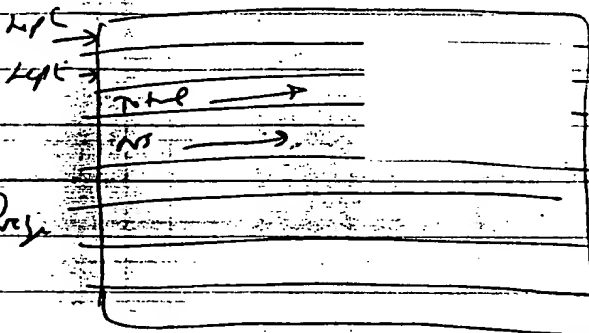
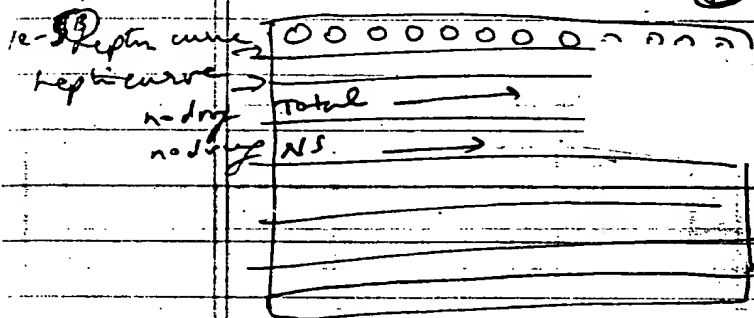
From Page No. _____

SPA binding on Oble-

Assay buffer: use leptin binding assay buffer + 1% DMSO.

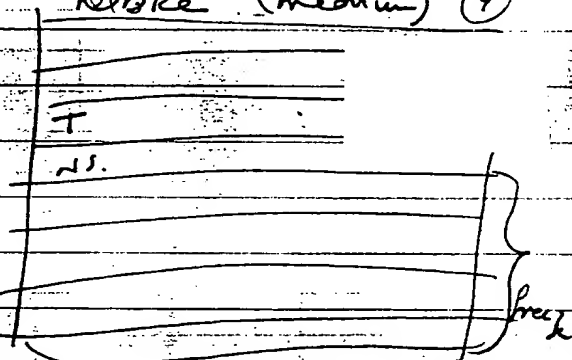
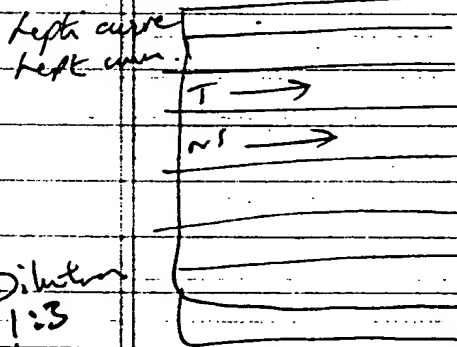
Oble (medium) ①

Mock (medium) ②



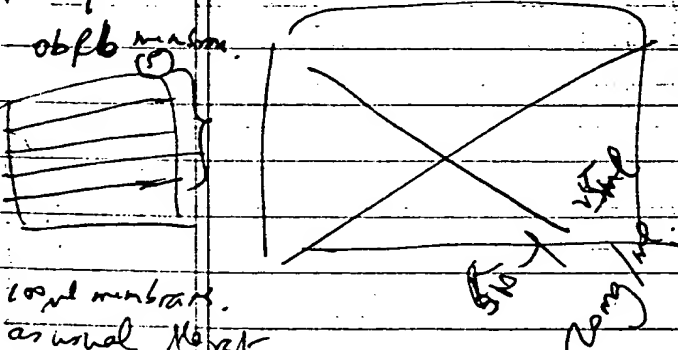
Oble (optimum) ③

Oble (medium) ④



Dilution
1:3

obfb membrane



count membrane
as usual H&E

run keep

rotor brought

Add to cell beads

100%
2% lipid
2% DMSO total

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TITLE Leptin Via Scintillation Proximity Assay Pr J c

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From Page No. _____

Protocol #: 13 10:54

Count Time (minutes): 1.00
Assay Type: CPM
Background Substrate: IPA Bkg
Outlier: 5.0 FLAG
Screening: OFF

Window A
Nuclide: I-125
Half Life (hours): 0.00
Multiplier: 1.0000
MDV Flag Limit: 0.00

Leptin
WARNING: BACKGROUND DATA

S# A:CPM A:ZERR B:CPM B:ZERR
1 123406 0.28 0.0
3 MISSING TUBE(S)

Table 1. Assay protocol

	Non-specific binding (NSB)	Zero standard (B ₀)	Standards	Test	Solvent Control
Buffer	-	50μl	-	-	-
Standards	50μl	-	50μl	-	-
Test Compound	-	-	-	50μl	-
Tracer	50μl	50μl	50μl	50μl	50μl
Test Solvent*	-	-	-	-	50μl
Cell preparation	50μl	50μl	50μl	50μl	50μl
WGA SPA beads	50μl	50μl	50μl	50μl	50μl
Cap tubes, shake overnight (16-24hr) at room temperature (15-30°C) Leave to settle for 1 hour					

Count in scintillation counter

* For example in organic solvents add the relevant solvent

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Date

Invented by

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$[^{125}\text{I}]$ Leptin - 0.1 nM (F) 100,000 cpm/μl

Dry plate

Non-specific 1×10^{-6} M (B) cold leptin.

MAKER 8 ml (^{125}I) Leptin

58.9 μCi / 0.1 ml 1840 Ci/mole

$$\frac{58.9 \mu\text{Ci} / 0.1 \text{ ml} \times 128 \text{ F}}{1,840 \times 10^9 \mu\text{Ci} / \text{mole}} = \frac{169}{1,840 \times 10^9} = 9.19 \times 10^{-8} \frac{\text{mole}}{\text{ml}}$$

$$9.19 \times 10^{-8} \frac{\text{mole}}{\text{ml}} \cdot V = 1 \times 10^{-9} \frac{\text{mole}}{\text{ml}} \cdot 8 \text{ ml}$$

$$V = 82 \mu\text{l}$$

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Date _____

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Date _____

Recorded by _____